

cyclooxygenase-1 (COX-1) and 12-lipoxygenase (12-LOX) leading to the production of a range of eicosanoids. Here we have determined the enzyme sources of the major prostanoid and HETE products released by platelets following activation by selective platelet agonists. To better model platelet activation we used either platelet rich plasma or whole blood within a platelet aggregometer, warmed and stirred at 1200rpm, and assessed whether HETEs originate from platelets or other blood cells. Furthermore, to investigate how the production of these eicosanoids is affected by anti-platelet therapy we examined the effects of the cyclooxygenase inhibitor, aspirin (100  $\mu$ M), with or without the addition of the P2Y<sub>12</sub> receptor blocker, prasugrel (3  $\mu$ M).

**Methods** Blood from healthy volunteers was collected into hirudin and incubated (30 min, 37°C) either intact or following preparation into platelet rich plasma (PRP) in the presence of collagen (30  $\mu$ g/ml), TRAP-6 (30  $\mu$ M) or vehicle, in a platelet aggregometer with stirring (1200rpm). Plasma was then separated from the samples and the levels of prostanoids and HETEs were determined by LC/MS/MS analysis. In some experiments, blood and PRP were pre-treated with aspirin (100  $\mu$ M), prasugrel (3  $\mu$ M) or aspirin+prasugrel.

**Results** LC/MS/MS analysis of collagen- and TRAP-stimulated PRP and blood showed large increases in the levels of thromboxane (TX) A<sub>2</sub>, prostaglandin (PG) E<sub>2</sub>, and PGD<sub>2</sub>, as well as 8-, 11-, 15- and 12-HETE compared to unstimulated samples. Interestingly, as well as inhibiting the production of TXA<sub>2</sub>, PGE<sub>2</sub> and PGD<sub>2</sub>, aspirin also strongly inhibited the productions of 11-HETE and 15-HETE by both PRP and blood. The production of 12-HETE was reduced by aspirin and prasugrel used in combination.

**Conclusions** LC/MS/MS analysis demonstrated that platelets are the main source of both prostanoids (TXA<sub>2</sub>, PGE<sub>2</sub> and PGD<sub>2</sub>) and HETEs (8-, 11-, 15- and 12) in blood exposed to platelet activators. Interestingly, in addition to abolishing the production of prostanoids, aspirin also blocked the production of 11-HETE and 15-HETE in both PRP and blood, indicating these HETEs are also formed through the activity of platelet COX-1. Aspirin in combination with prasugrel reduced, but did not abolish, the production of 12-HETE, consistent with this drug inhibiting the processes of platelet activation but not directly inhibiting platelet 12-LOX. It is possible these data may well help provide explanations for the beneficial effects of anti-platelet doses of aspirin in a range of cardiovascular diseases and cancers.

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#### PLATELET COX-1 SUPPORTS THE PRODUCTION OF BOTH PROSTANOIDS AND HETES

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**Background** Prostaglandins (PGs) and hydroxyeicosatetraenoic acids (HETEs) are synthesized from arachidonic acid (AA), which is released from the membrane phospholipids of cells through the activity of cytosolic phospholipase A<sub>2</sub> $\alpha$  (cPLA<sub>2</sub> $\alpha$ ). It is known that in platelets AA is metabolised through the enzymes